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ALGINATE-WHEY PROTEIN MICROENCAPSULATION FOR TARGET DELIVERY OF HYDROPHOBIC ANTIMICROBIALS TO THE PIG INTESTINE

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Introduction

- The emergence of antibiotic resistance in pathogenic bacteria has become a great concern over the years. The use of antimicrobial growth promoters (AGPs) has been banned in the EU since January 2006 (regulation EC/1831/2003) and is under debate in other countries. Therefore, there is a demand for alternatives to AGPs for food animal production.
- Carvacrol has long been used as a food preservative and has strong antimicrobial activity.
- Binding to feed ingredients or digesta and quick absorption of essential oil (EO) in the stomach and proximal small intestine diminishes its antimicrobial activity. Thus, encapsulation of EO is an approach for target delivery of EO to the distal intestine to achieve its antimicrobial function.
- The objective of this study was to develop novel encapsulation technology for target delivery of hydrophobic antimicrobial agents (carvacrol) to specific intestinal regions of pigs.

Materials and Methods

Materials: Carvacrol (98%), alginic acid sodium salt (A), low molecular weight alginate (L), whey protein (W), calcium chloride dehydrate.

Methods:

In Vitro

- Carvacrol was encapsulated in alginate-whey protein microcapsules by an emulsion-extrusion technique (Wang, et al., 2009).
- Response surface methodology was used to optimize encapsulation formulation and 20 formulas have been designed.
- The release profiles of the microcapsules were tested in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and ileal digesta (ID) from growing pigs at 37° C.

In Vivo

- Thirty purebred Yorkshire pigs (15 males, 15 females) from different litters and with initial BW of 11.9±0.9 kg were randomly grouped into three diet treatments: i) control: basal diet; ii) free oil: basal diet + 1500 ppm free carvacrol; iii) beads: basal diet + 1500 ppm encapsulated carvacrol (F8, fig.1).
- All piglets were slaughtered at 4, 5 and 6 h postprandially on day 8.
- Ti_2O_3 (0.2% w/w) was used as an indigestible marker in feed to monitor the apparent digestibility (disappearance) of carvacrol.
- Digesta from different intestinal regions was used for the analysis of the concentration and digestibility of carvacrol.

Results and Discussion

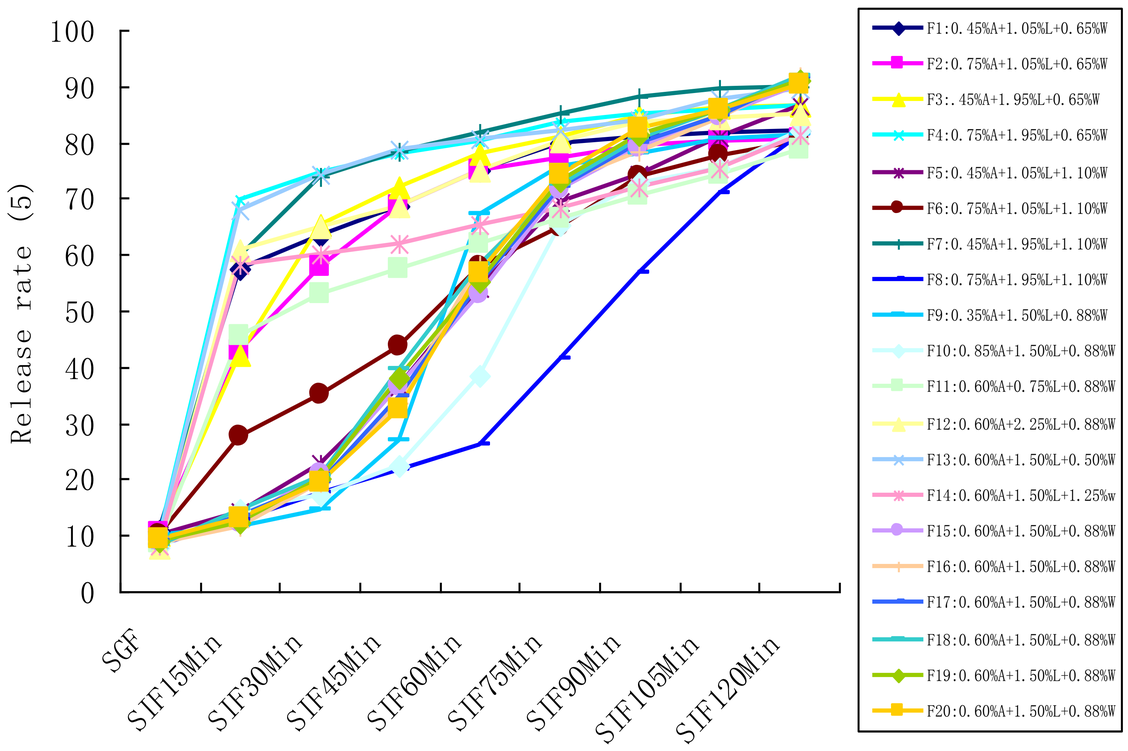

Figure 1. The release profile of 20 formulations in SGF and SIF

Table 1. Concentration and digestibility of carvacrol in different intestinal regions of pigs at different time points

Sections	Time points (hour)	Free oil		Beads	
		Concentration (ppm)	Digestibility (%)	Concentration (ppm)	Digestibility (%)
Stomach	4	$^{A}220.1\pm48.5^{a}$	$^{A}58.1\pm8.2^{a}$	$^{A}540.9\pm120.7^{b}$	$^{A}22.8\pm13.8^{b}$
	5	$^{A}229.9\pm77.5^{a}$	$^{A}63.2\pm10.0^{a}$	$^{B}647.1\pm42.2^{b}$	$^{A}25.0\pm8.5^{b}$
	6	$^{B}263.7\pm33.5^{a}$	$^{B}82.4\pm4.0^{a}$	$^{C}346.2\pm93.9^{b}$	$^{B}48.5\pm2.7^{b}$
Duodenum	4	132.3 ± 81.1^{a}	$^{A}66.2\pm21.1^{a}$	$^{A}835.4\pm365.7^{b}$	$^{A}28.7\pm9.1^{b}$
	5	126.9 ± 52.6^{a}	$^{A}70.6\pm12.2^{a}$	$^{B}437.6\pm24.2^{b}$	$^{B}31.8\pm12.7^{b}$
	6	234.0 ± 143.8	$^{B}95.1\pm0.2^{a}$	$^{C}298.4\pm8.2$	$^{C}53.8\pm8.7^{b}$
Jejunum	4	7.8 ± 6.7^{a}	98.5 ± 1.3^{a}	$^{A}273.0\pm54.8^{b}$	$^{A}43.6\pm14.4^{b}$
	5	20.8 ± 21.5^{a}	95.7 ± 3.0^{a}	$^{B}602.0\pm81.3^{b}$	$^{B}48.9\pm3.8^{b}$
	6	9.9 ± 3.7^{a}	99.9 ± 0.1^{a}	$^{C}121.9\pm19.9^{b}$	$^{C}75.7\pm3.8^{b}$
Ileum	4	10.9 ± 9.6^{a}	95.2 ± 0.5^{a}	$^{A}106.7\pm46.6^{b}$	80.7 ± 11.0^{b}
	5	8.9 ± 9.1^{a}	97.0 ± 1.6^{b}	$^{B}57.4\pm14.2^{b}$	83.0 ± 9.0^{b}
	6	13.3 ± 5.9^{a}	97.2 ± 3.0	$^{BC}45.0\pm17.3^{b}$	89.2 ± 7.8
Cecum	4	4.5 ± 1.5^{a}	99.6 ± 0.2^{a}	$^{A}31.7\pm4.9^{b}$	$^{A}97.0\pm0.9^{b}$
	5	8.1 ± 4.3^{a}	99.1 ± 1.0^{a}	$^{B}55.0\pm12.4^{b}$	$^{B}94.5\pm0.9^{b}$
	6	6.1 ± 0.8^{a}	99.9 ± 0.1^{a}	$^{BC}54.1\pm12.8^{b}$	$^{B}94.4\pm0.9^{b}$
Colon	4	5.5 ± 2.1^{a}	99.1 ± 1.9^{a}	$^{A}44.8\pm15.4^{b}$	$^{A}97.9\pm1.5^{b}$
	5	6.1 ± 1.9^{a}	99.4 ± 0.4^{a}	$^{B}66.6\pm9.7^{b}$	$^{B}94.9\pm0.5^{b}$
	6	3.4 ± 0.3^{a}	100.0 ± 0.1^{a}	$^{C}32.8\pm6.2^{b}$	$^{AC}97.6\pm0.5^{b}$

a, b, c: Values with different superscripts within same line differ significantly at $P < 0.05$.;
A, B, C: Values with different superscripts within same column differ significantly at $P < 0.05$.
Use singulars for Section, Time Point, Free Oil, and Bead in the table.

In Vitro

- The carvacrol retention rate (encapsulated carvacrol/added carvacrol) was over 98% and average carvacrol content of dry microcapsules was 71.8±0.5% (w/w).
- Depending on the encapsulation formulation, a wide spectrum of release profiles was obtained. All the microcapsules remained intact after 1 h incubation in SGF, but a small amount of carvacrol, ranging from 10.7±0.9% to l7.8±0.4% depending on the formulation, was released. More than 80% of encapsulated carvacrol was completely released within 2 h incubation in SIF (Fig. 1). .
- When incubated with ID, no intact microcapsule was found after 3.5 h.

In Vivo

- Cumulative digestibility of free carvacrol from the samples collected at 4, 5, and 6 h were 58.1±8.2%, 63.2±10.0%, 82.4±4.0% in the stomach and 66.2±21.1%, 70.6±12.2%, 95.1±0.2% in the duodenum, respectively.
- In contrast, encapsulation reduced the cumulative digestibility in the stomach to 22.8±13.8%, 25.0±8.5%, and 48.5±2.7%, and in the duodenum to 28.7±9.1%, 31.8±12.7% and 53.8±8.7%, respectively, which were significantly lower than the values of free carvacrol (P<0.0001, P<0.0001, P=0.0011 for 4, 5, 6 h, respectively). The cumulative digestibility of carvacrol were 43.6±14.4%, 48.9±3.8% and 75.7±3.8% in the jejunum for encapsulated carvacrol treatment. For free carvacrol, the digestibility of carvarol were 98.5±1.3%, 95.7±3.0% and 99.9±0.1% in jejunum.

Conclusion

The current study indicates that microencapsulation is a potential tool to increase the delivery of hydrophobic antimicrobial agents to the lower region of pig intestine to protect their antimicrobial activity. More studies are required to further improve the encapsulation technique for maximizing the delivery of carvacrol to the lower intestine region.

Reference

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